



Efficacy of sericea lespedeza hay as a natural dewormer in goats: Dose titration study

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ABSTRACT

Gastrointestinal nematodes (GIN) parasitism is the greatest threat to economic sheep and goat production in the southern USA, and there is widespread prevalence of GIN resistance to broad-spectrum anthelmintics in this region. A natural alternative for controlling GIN in small ruminants is feeding hay of sericea lespedeza [SL, *Lepedeza cuneata* (Dum.-Cours., G. Don)], a perennial warm-season legume high in condensed tannins. To determine the level of SL needed to reduce GIN infection, a confinement study was completed with 32 Spanish/Boer/Kiko cross yearling bucks offered one of four diets with 75% hay and 25% concentrate ($n = 8$, 2 pens/treatment, 4 goats/pen). The hay portion of each diet consisted of a combination of ground SL (0%, 25%, 50%, and 75% of the diet) and bermudagrass [BG, *Cynodon dactylon* (L.) Pers.; 75%, 50%, 25%, and 0% of the diet]. The bucks were allowed to acquire a natural GIN infection on pasture prior to moving to the pens. After a 3-week adjustment period in the pens, the goats were stratified by fecal egg count (FEC) and packed cell volume (PCV), randomly assigned to treatments and pens, and then fed the treatment diets for six weeks. During the experimental period, fecal and blood samples were collected from individual animals weekly to determine FEC and PCV, respectively. Adult worms from abomasum and small intestines were collected for counting and identification of species at slaughter. Goats fed SL hay at 25%, 50%, and 75% of the diet had 45.3% ($P = 0.2048$), 66.3% ($P = 0.0134$), and 74.5% ($P = 0.0077$) lower FEC than control animals (75% BG hay) after 21 days. The 50% and 75% SL goats had 84.6% ($P = 0.0625$) and 91.9% ($P = 0.0340$) lower FEC than controls by day 42. The 75% SL-fed goats tended to have higher ($P = 0.0624$) PCV and had fewer ($P = 0.035$) abomasal worms than control animals, while PCV and adult worm numbers of the 50% and 25% SL goats were not different from controls. The optimum level of SL hay in the diet for reducing worm numbers of small ruminants appears to be 75%, whereas 50–75% SL reduces FEC, which could lead to reduced larval infection on pasture.

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1. Introduction

Goat production is a growing industry in the United States (US), but economic production is constrained by losses due to infection with gastrointestinal nematodes (GIN), particularly the blood-feeder *Haemonchus contortus* (Miller, 1996). Since their introduction in the 1960s, broad-spectrum synthetic

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anthelmintics have been the primary defense against GIN infection in small ruminants in the US and around the world, but this strategy is no longer viable due to widespread development of anthelmintic resistance in sheep and goat parasites (Pritchard, 1994; Bath, 2006; Waller et al., 1996). Recent reports from the southern US (Zajac and Gipson, 2000; Terrill et al., 2001; Mortensen et al., 2003; Kaplan et al., 2007) indicate that anthelmintic resistance in goats has become highly prevalent in this region.

A natural alternative to synthetic anthelmintics for controlling GIN infection in animals is the use of tannin-rich plants. Grazing forages high in condensed tannins (CT) has been shown to reduce the number of parasite eggs in sheep and goat feces (Niezen et al., 1995; Min and Hart, 2003; Paolini et al., 2003a), and there have been recent reports of anthelmintic activity of CT forages fed as dried hay (Paolini et al., 2003b; Shaik et al., 2004, 2006; Lange et al., 2006). Reduced FEC and worm burden have been reported for goats fed hay of the CT forage sericea lespedeza [SL, *Lepedeza cuneata* (Dum.-Cours., G. Don)], a warm-season perennial legume that is well adapted to the southern USA (Lange et al., 2006; Shaik et al., 2006). When fed as the primary component of the diet (75% of feed offered), SL hay reduced fecal egg count (FEC) in goats by 80% compared with animals fed bermudagrass [BG, *Cynodon dactylon* (L.) Pers.] hay (Shaik et al., 2004, 2006). The anthelmintic efficacy of lower amounts of SL hay in the diet of small ruminants has not been determined. The purpose of the current study was to determine if significant anthelmintic effects could be achieved by feeding SL hay to goats at 25% and 50% of the diet offered.

2. Materials and methods

2.1. Experimental design and protocol

The Fort Valley State University (Fort Valley, GA) Institutional Animal Care and Use Committee reviewed and approved all husbandry practices and experimental procedures used in this study. A confinement feeding study with 32 yearling meat goat bucks (Spanish × Boer × Kiko cross, 17.8 ± 4.1 kg) was completed at the Fort Valley State University Agricultural Research Station from October through November, 2005. Prior to starting the trial, the goats acquired a natural GIN infection by grazing (continuously stocked, 10 goats/hectare) perennial summer grass pasture (primarily BG and bahiagrass, *Paspalum notatum* Flugge) for approximately 6 months. After being moved into feeding pens, all the goats were fed ground BG hay (75% of feed offered) plus concentrate (25% of feed offered) during a 3-week adjustment period, during which feces and blood samples were collected from individual animals weekly for FEC and packed cell volume (PCV) analysis, respectively. At the end of this period (0 sample time), the bucks were randomly assigned to diet and pen based upon FEC and PCV ($n = 8$, 2 pens/treatment, 4 goats/pen). No animals were dewormed during this study.

2.2. Experimental diets

The goats were offered one of four diets of 75% hay (combination of SL and BG) and 25% commercial goat

concentrate (16% CP, Purina Mills, LLC, St. Louis, MO) for 6 additional weeks. The hay portions of the dietary treatments were 75% BG (control), 75% SL, 50% SL + 25% BG, and 25% SL + 50% BG. All diets were offered on a 3.5% BW as-fed basis, and the goats were given access to water *ad libitum*.

2.3. Analysis of hay samples

Samples of SL and BG hays were ground to pass a 1-mm screen using a Cyclotec sample mill (Foss, Eden Prairie, MN) and analyzed for nitrogen content using a carbon-nitrogen analyzer (Vario Max, Elementar Americas Inc., Mt. Laurel, NJ), with CP calculated as $N \times 6.25$ (AOAC, 1990). Ground SL hay was analyzed for extractable, protein-bound, and fiber-bound CT content by the Terrill et al. (1992) method using purified SL tannin as the standard.

2.4. Sampling procedures and analysis

All FEC determinations were made on fresh feces. Eggs per gram (EPG) of feces were counted using a modified McMaster procedure (Whitlock, 1948) and PCV was determined using a Marathon 6 K micro-hematocrit centrifuge and reader (Fisher Scientific, Pittsburgh, PA). Fecal egg count reduction compared to control was determined as follows:

$$\% \text{FEC reduction} = \frac{\text{control EPG} - \text{treatment EPG}}{\text{control EPG}} \times 100.$$

Fecal cultures were prepared 2 weeks after the start of the trial on pooled samples from each GIN-infected treatment group using a modified Baermann's procedure as described by Terrill et al. (2004). For each pooled sample, whole fecal pellets (10 g) were crushed slightly, moistened with distilled/deionized water, placed in a small moisture chamber for 14 days at room temperature, after which larvae were retrieved using a modified Baermann apparatus, and then counted and identified to species using a Swift M4000-D light microscope (Swift Optical Instruments, San Antonio, TX). The percentage of *H. contortus* larvae present and percentage of total larvae recovered were calculated using the following formula:

$$\% \text{Larval recovery} = \frac{\text{larvae/g}}{\text{eggs/g}} \times 100.$$

2.5. Recovery and counting of adult nematodes

Adult GIN from abomasum and small intestines were recovered, counted, and identified to species using a Leica Zoom 2000 phase contrast microscope (Leica Microsystems Inc., Chicago, IL) as described by Shaik et al. (2006). Prior to counting, the contents of the abomasum and small intestines for each animal were washed into plastic buckets, brought up to 3 L with water, mixed thoroughly, and then subsampled twice (2 aliquots of 150 mL each). The adult GIN were preserved by adding 100 mL of 10% buffered formalin solution (Sigma-Aldrich, St. Louis, MO, USA) to each container.

2.6. Statistical analyses

Fecal egg count and PCV data were analyzed as the repeated measures analysis in a completely randomized design using the mixed model procedure of SAS (1996). Variables included in the model were treatment, time, and the interaction. Adult GIN data were analyzed as a completely randomized design using the GLM procedure of SAS. The FEC and adult GIN data were log-transformed prior to statistical analysis to normalize the data. When treatment effects were different at $P < 0.05$, means were separated using LSD test. Fecal egg count and adult GIN data are reported as least squared means, with statistical inferences based upon log-transformed data analysis. Because larval cultures were made from pooled fecal samples for each treatment group, percentage larval recovery and percentage *H. contortus* in recovered larvae data were not subjected to statistical analysis. These data are presented as arithmetic means \pm SD from 2 replicates/treatment group.

3. Results

3.1. Hay analyses

Crude protein contents of SL and BG hays were 9.3% and 10.2%, respectively, on an as-fed basis. Extractable, protein-bound, fiber-bound, and total CT of SL hay was 6.2%, 3.1%, 0.5%, and 9.8%, respectively.

3.2. Fecal egg counts

The treatment and time main effects were significant ($P = 0.0287$ and $P = 0.0056$, respectively) for FEC data. There were little differences in FEC among treatment groups during the first 14 days of the experimental period (Table 1).

Table 1

Nematode egg count in feces of parasitized goats fed four different levels of ground sericea lespedeza (SL) or bermudagrass (BG) hay and 25% of concentrates.

Diet, %		Days after initiation of dietary treatments						
SL	BG	0	7	14	21	28	35	42
Nematode egg count per gram feces ^a								
0	75	2033 \pm 576a ^b	2900 \pm 671a	2100 \pm 806a	3383 \pm 518b	1717 \pm 423a	1667 \pm 540ab	3516 \pm 793b
25	50	2140 \pm 446a	3200 \pm 520a	1930 \pm 625a	1850 \pm 401ab	1260 \pm 328a	2410 \pm 418b	2340 \pm 614ab
50	25	1770 \pm 446a	2680 \pm 520a	1420 \pm 625a	1140 \pm 401a	930 \pm 328a	970 \pm 418a	540 \pm 614ab
75	0	1783 \pm 407a	2042 \pm 475a	1517 \pm 570a	967 \pm 366a	667 \pm 299a	858 \pm 382a	225 \pm 561a

^a \pm Standard error.

^b Column means with unlike letters differ ($P < 0.05$).

Table 2

Blood packed cell volume of parasitized goats fed four different levels of ground sericea lespedeza (SL) or bermudagrass (BG) hay and 25% of concentrates.

Diet, %		Days after initiation of dietary treatments						
SL	BG	0	7	14	21	28	35	42
Blood packed cell volume ^a								
0	75	16.6 \pm 2.0a ^b	18.7 \pm 2.3a	21.4 \pm 2.5ab	20.7 \pm 2.5a	19.3 \pm 2.2a	18.8 \pm 2.2a	19.5 \pm 2.7ab
25	50	17.0 \pm 1.8a	17.2 \pm 2.1a	17.3 \pm 2.3a	16.8 \pm 2.2a	17.7 \pm 2.0a	18.1 \pm 2.0a	18.1 \pm 2.5a
50	25	18.0 \pm 1.8a	16.3 \pm 2.1a	19.0 \pm 2.3ab	19.9 \pm 2.2a	19.3 \pm 2.0a	22.3 \pm 2.0a	22.8 \pm 2.5ab
75	0	20.0 \pm 2.6a	21.0 \pm 3.0a	24.7 \pm 3.2b	23.3 \pm 3.2a	24.2 \pm 2.9a	25.3 \pm 2.9a	28.3 \pm 3.5b

^a \pm Standard error.

^b Column means with unlike letters differ ($P < 0.05$).

After 21 days, goats fed SL hay at 25%, 50% and 75% of the diet had 45.3% ($P = 0.2048$), 66.3% ($P = 0.0134$), and 74.5% ($P = 0.0077$) lower FEC than control animals (Table 1). There was little or no reduction in FEC compared to control for the 25% SL goats for the duration of the trial, but the 50% and 75% SL-fed animals were 84.6% ($P = 0.0625$) and 91.9% ($P = 0.0340$) lower than controls by day 42.

3.3. Blood packed cell volume

For PCV data, the treatment \times time interaction was significant ($P = 0.0263$). Packed cell volume in SL-fed goats was not different from the control animals during the first 28 days of the experiment (Table 2). By day 35, although not significantly different, PCV of the 75% SL-fed goats tended to be higher than the 25% SL and control animals ($P = 0.0562$; $P = 0.0914$, respectively), while on day 42, the 75% SL-fed goats were higher ($P = 0.0289$) than the 25% SL and tended to be higher ($P = 0.0624$) than control animals. There were no differences between PCV values of the 50% SL and 25% SL goats and control animals throughout the trial.

3.4. Percentage larval development in fecal cultures

The percentage *H. contortus* larvae recovered from fecal cultures was similar between all the treatment groups, ranging from 35.7% to 46.9%. The overall percentage of eggs that developed into larvae varied based upon level of SL hay in the diet, with 38.5%, 56.7%, 56.8%, and 75.4% development in goats fed 75%, 50%, 25%, and 0% SL in their diet.

3.5. Adult nematodes

Including 25%, 50%, and 75% SL in the diet of goats reduced total number of abomasal worms by 49.5% ($P = 0.4989$), 45.4% ($P = 0.6953$), and 74.0% ($P = 0.035$),

Table 3

Number of adult gastrointestinal nematodes (GIN) in the abomasum and small intestine of parasitized goats fed four different levels of ground sericea lespedeza (SL) or bermudagrass (BG) hay and 25% of concentrates.

Diet, %		Number of adult GIN ^a			
		Abomasum		Small intestine	
SL	BG	<i>Haemonchus contortus</i>	<i>Teladorsagia circumcincta</i>	<i>Trichostrongylus colubriformis</i>	Total GIN
0	75	2264 ± 433a ^b	122 ± 79a	2808 ± 1505ab	5195 ± 1686ab
25	50	944 ± 411a	262 ± 75b	7036 ± 1427c	8242 ± 1600b
50	25	1278 ± 459a	23 ± 84a	1720 ± 1596a	3020 ± 1788a
75	0	570 ± 459a	50 ± 84a	3610 ± 1596bc	4230 ± 1788ab

^a ±Standard error.

^b Column means with unlike letters differ ($P < 0.05$).

respectively, compared with control animals (Table 3). Total number of *H. contortus* in the abomasum was 58.3%, 43.6%, and 74.8% lower in 25% ($P = 0.0946$), 50% ($P = 0.2762$), and 75% SL-fed goats ($P = 0.0501$), respectively. The number of total GIN was not different between control animals and goats fed 25%, 50% and 75% SL, while the 25% SL animals were significantly higher than controls in number of *Teladorsagia circumcincta* ($P = 0.0363$) and *Trichostrongylus colubriformis* ($P = 0.0195$).

The abomasal worms identified in control animals were primarily *H. contortus* (94.9%), with some *T. circumcincta* (5.1%), and all of the small intestinal worms were identified as *T. colubriformis*. Overall, the goats' GIN infections (based upon control animals) were 54.1% *T. colubriformis*, 43.6% *H. contortus*, and 2.3% *T. circumcincta*.

4. Discussion

Diets with 50% and 75% SL hay reduced FEC in goats, while reduction in abomasal worms only occurred at the 75% SL level. Positive effects of feeding SL hay to parasitized animals at 75–80% of the diet have been reported previously for both sheep and goats (Lange et al., 2006; Shaik et al., 2004, 2006). Shaik et al. (2006) reported an 80% reduction in FEC in goats fed SL compared with BG hay after 1 week. Lange et al. (2006) reported a similar immediate drop in FEC in sheep fed SL hay compared with BG hay. Comparable reductions in FEC were observed for the 50% and 75% SL-fed goats in the current experiment, but the effect was not as immediate. The reason for this delay in the effect on egg production is not clear, but may be related to the time of year the current trial was completed. The previous experiments were completed during the summer months, while the current trial was completed during October and November. The primary GIN infecting goats and sheep in summer is *H. contortus* throughout much of the USA, while other nematode species, such as *T. circumcincta* and *T. colubriformis* often increase relative to *H. contortus* in fall and winter. In the current investigation, the infection in control animals was over half *T. colubriformis* (54%), while *H. contortus* was only 44%. As SL has a greater suppressing effect on *H. contortus* than other GIN species (Shaik et al., 2006), higher numbers of non-*Haemonchus* worms may dampen the effect on FEC reduction.

Although not significant, the increase ($P = 0.0624$) in PCV of the 75% SL hay-fed goats compared with control animals (75% BG hay diet) is higher than previous reports

for sheep and goats (Lange et al., 2006; Shaik et al., 2006). Despite differences relative to control animals, Shaik et al. (2006) reported a slight decline in PCV in SL-fed animals during the experimental period. In the current trial, PCV of the highest SL group increased from 20.0% to 28.3%, while the 50%, 25%, and 0% SL groups all had slight increases by the end of the trial (Table 2). The increased PCV for all treatments was likely due to an overall worm burden dominated by *T. colubriformis* rather than *H. contortus* due to cooling temperatures during the period of the study as discussed previously. Despite this trend, the 75% SL group had lower total numbers of adult *H. contortus* ($P = 0.0501$) compared with control animals, which led to a greater increase in PCV in these animals. The 50% and 25% SL-fed goats also had fewer adult *H. contortus* compared with controls, but the differences were not significant and apparently not enough to affect PCV values.

The percentage reduction in *H. contortus* and total abomasal worms due to presence of SL in the diet ranged from 45% to 55% for 25% and 50% SL, and approximately 75% for the 75% SL-fed goats (Table 3). The abomasal worm reduction levels for the 75% SL goats were similar to previously published results for both sheep and goats fed SL hay at this rate (Shaik et al., 2006; Lange et al., 2006). Despite non-significant effects in 50% and 25% SL-fed goats ($P = 0.2762$ and $P = 0.0946$, respectively), *H. contortus* numbers in the abomasum were reduced by approximately half in these animals. Shaik et al. (2006) reported reductions in *T. circumcincta* and *T. colubriformis* in goats fed SL at 75% of the ration compared with control animals, but the reductions were much lower than with *H. contortus*. The current study confirms this differential effect of dietary SL on GIN species, but the number of *T. colubriformis* was either similar or higher in SL-fed goats compared with those fed no SL in the diet. Athanasiadou et al. (2001) reported a differential effect of Quebracho CT extracts added to sheep diets on worm burdens. Intestinal worms (*T. colubriformis*) were reduced in a dose-dependent manner (greater reduction at 8% CT than 4% CT added), while abomasal worm numbers (*H. contortus* and *T. circumcincta*) were not affected.

The reason for differential effects of dietary CT on different GIN species is probably related to CT activity in the gastrointestinal tract. Previous studies with CT-containing forages have demonstrated that CT form temporary complexes with dietary proteins in the neutral pH of the rumen, but are released from these complexes in the acid pH of the abomasum, increasing their availability

to react with other compounds (Barry et al., 2001). Once leaving the abomasum, CT reactivity is again reduced due to increasing pH of the small intestine (Jones and Mangan, 1977). Direct interaction of CT with adult *H. contortus* in the abomasum would account for this differential effect on GIN species. In a recent report by Brunet and Hoste (2006), exsheathment of *H. contortus* and *T. colubriformis* infective larvae (L₃) were differentially affected by the CT monomers prodelphinidin and procyanidin in *in vitro* tests, with the differences related to different protein compositions of the sheaths. In an *in vivo* test, Brunet et al. (2007) reported that consuming the high-CT legume sainfoin (*Onobrychis viciifolia* scop.) delayed exsheathment of *H. contortus* larvae in a dose-dependent manner. In a follow-up study, Brunet et al. (2008) reported differential responses in the ability of *H. contortus* and *T. colubriformis* L₃ to penetrate simulated abomasal mucosa due to the action of the same CT monomers and CT extracts from sainfoin.

5. Conclusions

The optimum level of SL hay in the diet for reducing both FEC and adult worm numbers in the abomasum of small ruminants appears to be 75%, with reduced egg production at the 50% SL level. Although feeding SL at 50% of the diet would be beneficial for possible reduction of pasture infection with GIN larvae, it appears that a higher level of dried SL is needed to kill adult worms. Additional dose titration studies with SL ground or pelleted hay fed to sheep and goats with a primary *H. contortus* infection are needed, but the current investigation provided further evidence of the potential of SL hay as a natural supplement to or replacement for chemical anthelmintics.

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